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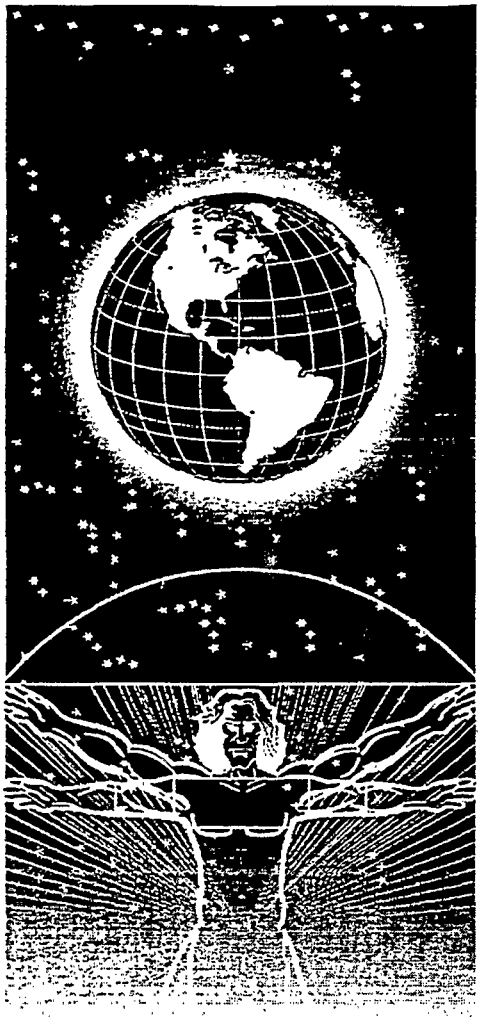

UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

STABILITY AND CONCENTRATION VERIFICATION OF AMMONIUM PERCHLORATE DOSING SOLUTIONS

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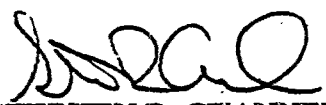
The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR



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13. ABSTRACT *(Maximum 200 words)*
Stability and concentration verification was performed for the ammonium perchlorate dosing solutions used in the on-going 90-Day Oral Toxicity Study conducted by Springborn Laboratories, Inc. (SLI Study No. 3455.1) and the Neurobehavioral Development study conducted by Argus Research Laboratories, Inc. (SS No. 7757A210-1096-25F). A sensitive and selective ion chromatography (IC) method for the analysis of perchlorate (ClO4-) and nitrate (NO3-), a possible interference anion, was developoed to support these studies. The method development, and validation data more than sufficiently demonstrated that the IC method was capable of detecting both perchlorate and nitrate at 0.005 ug/mL (5 ppb) in reagent water with excellent accuracy and precision. Ion chromatographic analysis of the stability solutions showed that under controlled room temperature, relative humidity and light intensity, ammonium perchlorate was stable in reagent water for at least 109 days. The concentrations of the ammonium perchlorate dosing solutions (0.05 to 200 ug/mL) were verified by IC analysis to be within an acceptable range of +/- 10%.

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PREFACE

The research described in this report began in August 1997 and was completed in January 1998 under the Department of the Air Force Contract No. F41624-96-C-9010. Lt Col Terry A. Childress served as Contract Technical Monitor for the United States Air Force, AFRL/HEST. Darol E. Dodd, Ph.D. served as Program Manager for the ManTech/Geo-Centers Joint Venture Contract.

LIST OF ABBREVIATIONS

NH_4^+	ammonium cation
NH_4ClO_4	ammonium perchlorate
Avg.	average
cm	centimeter
CAS NO.	chemical abstract services registry number
CV or % CV	coefficient of variation or percent coefficient of variation
Conc.	concentration
α	confidence factor
F crit	critical value of the *F* ratio
^oC	degrees Centigrade
^oF	degrees Fahrenheit
df	degrees of freedom
fc	footcandle
g	gram
L	liter
MS	mean square
MDL	method detection limit
µg	microgram
µl	microliter
µM	micromolar or micromole/liter
µmol	micromole
µS	micro-siemen
mg	milligram
mL	milliliter
mm	millimeter
MΩ	milli-ohm
min	minute

M	molarity
mol	mole
ng	nanagram
NO_3^-	nitrate anion
ND	non-detect
ANOVA	one-way analysis of variance
oz.	ounce
ppb	parts per billion
ppm	parts per million
ClO_4^-	perchlorate anion
PQL	practical quantitation limit
p-value	probability value
QA	quality assurance
QC	quality control
n	sample size
Std. Dev.	standard deviation
SS	sum of squares
F	the *F* ratio, test for homogeneity of variance
F crit	the F *value*
UV	ultraviolet

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STABILITY AND CONCENTRATION VERIFICATION OF AMMONIUM PERCHLORATE DOSING SOLUTIONS

SECTION I: INTRODUCTION

Ammonium perchlorate (NH_4ClO_4, CAS NO. 7790-98-9) is a white, crystalline salt that readily dissociates in water. The solubility of NH_4ClO_4 in water at 20°C is 107.44 mg/mL. NH_4ClO_4 is a powerful oxidizer (Class 1.1, Department of Transportation) and it has been used extensively by the Department of Defense in solid propellant mixtures for rockets, missile engines and munitions. [1] Perchlorate is not regulated in the U.S. under the federal Safe Drinking Water Act and it was once used pharmaceutically to treat hyperthyroidism (Grave's disease) [2-4]. However, since finding greater than 0.005 µg/mL (5 ppb) of perchlorate in several western U.S. municipalities, DoD has initiated several research studies on perchlorate. [5-10]

We provided a stability study and a concentration verification of the ammonium perchlorate dosing solutions used in the 90-Day Oral Toxicity study conducted by Springborn Laboratories and the Neurobehavioral Development study conducted by Argus Research Laboratories. A sensitive ion chromatography (IC) method for the analysis of perchlorate (ClO_4^-) and nitrate (NO_3^-), a possible interference anion, was developed to support these studies.

SECTION II: METHODS AND MATERIALS

Compliance Statement

The study entitled "Stability and Concentration Verification of Ammonium Perchlorate Dosing Solutions" was conducted to be in compliance with the Environmental Protection Agency's Good Laboratory Practices Standards, 40 CFR 792.

Test Materials

Primary ammonium perchlorate (lot # 03907LF) and ammonium nitrate (lot # 09016AR) standards were purchased from Aldrich Chemical Company (St. Louis, MO). Secondary ammonium perchlorate (lot # K15G11) and ammonium nitrate (lot # 22141) check standards were purchased from Alpha Chemical Company (Ward Hill, MA). Test materials were used without further purification.

Reagents

Sodium hydroxide (45 mM) was prepared by dissolving 1.8 g of NaOH in 1 L 55:45 reagent water and HPLC grade methanol. Sodium hydroxide was purchased from Aldrich Chemical Company (Milwaukee, WI). HPLC grade methanol was purchased from CORCO Chemical Company. Type I reagent water (18.0 to 18.3 MΩ-cm) was collected from a Barnstead Model D4751 Ultra-Pure water system.

Calibration Standards

Ammonium perchlorate and ammonium nitrate stock standard solutions at 50 mg/mL were prepared gravimetrically (Metler Model PE-360 analytical balance, ± 0.0001 g) from pure neat standards. 1000 µg/L working standard solutions were prepared from the individual stock standard solutions. From the working standard solutions, calibration standards at 0.05, 0.10, 0.50, 1.00, 2.00, 5.00, 10.0, 20.0, 50.0, 100 and 200 µg/mL were prepared by serial dilution.

Analytical Method

Ion chromatography was performed on a Dionex DX-300 High Performance Liquid Chromatograph with a Dionex CDM-3 conductivity detector. An ASRS-II anion suppresser, operating in auto suppression-external water mode, was used. The system included a Dionex AI 350 autosampler. All data were collected using Dionex AI-450 software. Dionex IonPak AS-11 ion chromatography column (4.0 x 250 mm), Dionex ATC-1 anion trap column and Dionex AG-11 guard column (4.0 x 50 mm) were used to perform anion analysis. The mobile phase, consisting of 45 mM NaOH in 55:45 water:methanol, was set at 1 mL/min flow rate. The injection loop volume was 50 µL, and the regenerant flow rate was 10 mL/min. Analysis was performed at 30°C.

Perchlorate Stability Analysis

Stock perchlorate solution (50 mg/mL) was prepared gravimetrically by adding 25 g of ammonium perchlorate to a 500 mL volumetric flask and bringing to volume with Type I laboratory reagent water. From the 50 mg/mL stock standard solution, 4 L perchlorate stability solutions at 0.05 and 200 µg/mL were prepared in a polyethylene carboy (Consolidated Plastics Co., Twinsberg, OH. Cat. # 22788LH) by serial dilution. The concentrations of the 0.05 and 200 µg/mL stability solutions were verified by ion chromatography. For each perchlorate solution, 200 mL aliquots were transferred to four 16-oz. clear French square bottles (Ancare Corp., Bellmore, NY, Cat. # FS-101) and three 300 mL amber French square bottles (All-Pack, Bridgeville, PA, Cat #7934). Each bottle was capped with a double-bearing metal spout, sealed with a rubber septum, and transferred to a rodent cage. The concentration of the stability solutions was monitored by ion chromatography on days 7, 15, 36, 50, 61, and 109 from the date of preparation.

To simulate perchlorate exposure to light during a normal animal testing study, a rack housing 14 rodent cages complete with bedding and feeder, was centered between light sources in an animal room. Water bottles containing stability solutions were placed on the cages. The temperature and relative humidity of the animal room was maintained at 70 to 72°F and 60 to 65% relative humidity. The light/dark cycle was set at 12 hour intervals. The animal cages were

rotated daily. On days 10, 27, 41, and 54, the light intensity of the animal room was measured with a Litemate III model 504 light meter (Photo Research Division, Kollmorgen Instrument Corporation, Chatsworth, CA).

Concentration Verification

Dose formulations were prepared by Springborn Laboratories and Argus Research Laboratories. Triplicate samples (2 mL each) were taken from the prepared formulations on the day of preparation. One of each triplicate was retained at the testing laboratories as a backup sample. Two samples were shipped on ice to AFRL/HEST by overnight delivery. To avoid cross contamination, the samples were immediately stored in a dedicated 4 to 6°C refrigerator upon receipt. Within 36 hours of arrival, the samples were analyzed by ion chromatography for nitrate and perchlorate.

SECTION III: RESULTS

Method Development and Validation

Chromatographic conditions were optimized and described in Section II. Baseline noise was kept minimal. The system was equilibrated to produce a background conductance less than 1.9 µs. A system blank, deionized water, established the baseline and confirmed the lack of contamination in the system. A typical ion chromatogram of 5 µg/mL nitrate and perchlorate standards is shown in Figure 3.1. The order of elution was established by injecting each standard separately, at 10 µg/mL. The retention times for nitrate and perchlorate were approximately 2.2 and 9.4 min, respectively. Both peaks were well-resolved. The nitrate peak was relatively sharper than that of the perchlorate peak. The peak-width at half height for nitrate was 0.3 minutes and for perchlorate, 0.5-0.6 minutes.

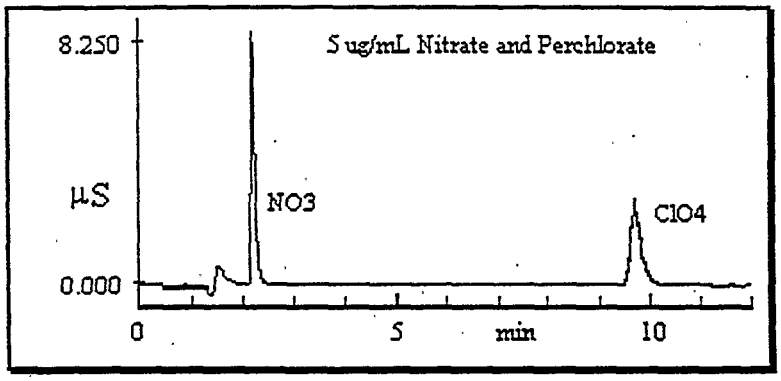

Figure 3.1. An ion chromatogram of 5 µg/mL nitrate and perchlorate.

The perchlorate peak could be sharpened by using straight 100 mM sodium hydroxide in reagent grade water as mobile phase rather than using 45 mM sodium hydroxide in 45:55 reagent grade water: HPLC grade methanol. However, with a straight 100 mM sodium hydroxide mobile phase, the nitrate peak is un-retained and it runs into the water/injection peak.

The method detection limit data for nitrate and perchlorate are shown in Table 3.1. Eight replicates of 0.05 µg/mL standards containing both nitrate and perchlorate were prepared. The

0.05 µg/mL replicates were analyzed over 72 hours. Per guidelines and procedures set forth in Code of Federal Regulations 40, Chapter 1, Pt. 136, Appendix B, [12] the calculated method detection limit (MDL) for both nitrate and perchlorate is 0.005 µg/mL (5 ppb). The appropriate Student t-test value for eight samples (n= 8, degree of freedom = 7) at the 99% confidence limit is 2.998. The relative percent recovery for nitrate and perchlorate were 101 and 100%, respectively. For both nitrate and perchlorate, the signal to noise ratio at 0.005 µg/mL (5 ppb) is greater than 3, and the chromatograms of perchlorate and nitrate at the detection limit are shown in Figures 3.2 and 3.3, respectively. Both chromatograms show that the perchlorate and nitrate peaks are well resolved even at the detection limit. The calculated on-column limit is 0.25 ng (0.005 µg/mL * 50 µL = 2.5×10^{-4} µg) and the practical quantitation limit at 10 times the MDL is 0.05 µg/mL. The lower and upper confidence limits, set at 95% confidence limit, are 0.0036 and 0.011 µg/mL.

Table 3.1. MDL data for perchlorate and nitrate analysis by ion chromatography

Perchlorate			Nitrate		
Data Points	**Area Count**	**Conc. (µg/mL)**	**Data Points**	**Area Count**	**Conc. (µg/mL)**
1	212034	0.050	1	480855	0.048
2	211140	0.050	2	517693	0.051
3	221194	0.052	3	521485	0.051
4	227660	0.054	4	517938	0.051
5	210420	0.050	5	528041	0.052
6	213280	0.050	6	470344	0.048
7	209608	0.050	7	499425	0.049
8	206890	0.049	8	505112	0.050
Avg. Conc. (µg/mL)		0.051	Avg. Conc. (µg/mL)		0.050
Expected Conc. (µg/mL)		0.050	Expected Conc. (µg/mL)		0.050
% Recovery		101%	% Recovery		100%
Standard Deviation		0.002	Standard Deviation		0.002
MDL (µg/mL)		0.005	MDL (µg/mL)		0.005
PQL (10Xmdl)		0.05	PQL (10Xmdl)		0.05

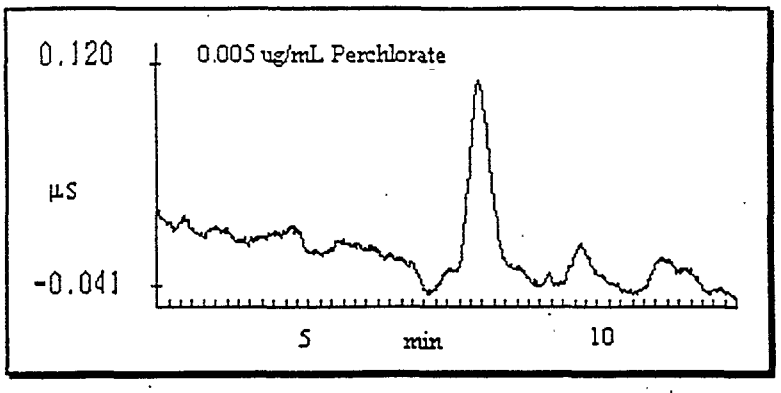

Figure 3.2. A typical ion chromatogram of 0.005 µg/mL perchlorate standard

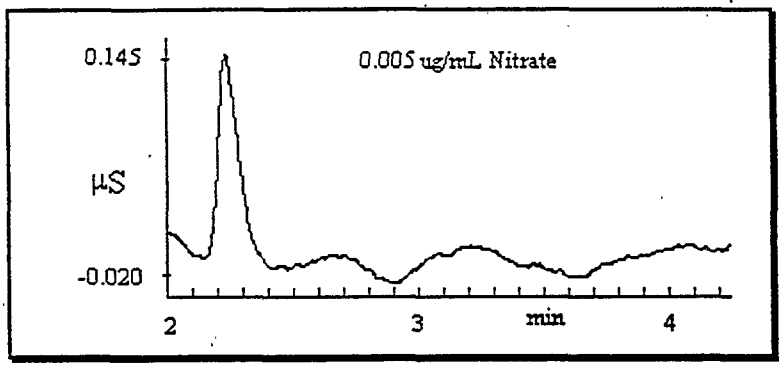

Figure 3.3. A typical ion chromatogram of 0.005 µg/mL nitrate standard

Calibration curves for nitrate and perchlorate were generated by plotting the concentrations of each standard against the peak area count obtained. The calibration curves are shown in Figure 3.4. In each case, the calibration curves were linear through the calibration range, from MDL to 40000 x MDL, over 5 orders of magnitude. For perchlorate, the calibration line was typically described by the equation Y = 5939330.55 X and nitrate, Y = 10247858.25 X. The correlation coefficient values were 0.9999 for both perchlorate and nitrate. The calibration curves were verified by nitrate and perchlorate standards purchased from a second source.

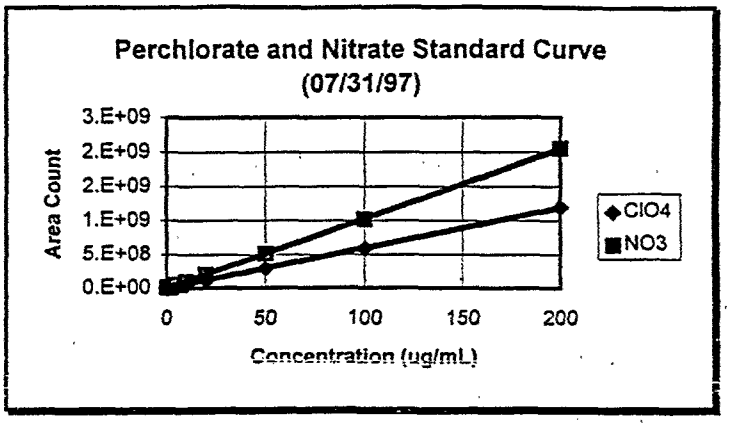

Figure 3.4. Typical calibration curves for perchlorate and nitrate

The intra- and inter-day method variabilities for perchlorate and nitrate were measured and expressed as percent coefficient of variation (Table 3.2). The average intra- and inter-day variability was less than 10% coefficient of variation for all concentration levels and did not show concentration dependence.

Table 3.2. Intra- and Inter-day variabilities for perchlorate and nitrate

	Perchlorate		Nitrate	
Concentrations (µg /mL)	**% Intra-day Variability**	**% Inter-day Variability**	**% Intra-day Variability**	**% Inter-day Variability**
n	**2**	**4**	**2**	**4**
0.05	0.42	7.99	0.00	5.38
0.10	2.83	3.73	2.22	8.86
0.50	8.14	2.86	1.85	0.69
1.00	8.74	4.98	1.06	1.19
2.00	0.81	9.81	2.12	2.32
5.00	1.86	4.34	1.82	5.99
10.0	0.13	3.02	0.58	4.26
20.0	1.87	0.93	0.95	0.51
50.0	1.72	0.52	0.10	1.99
100	1.54	0.03	0.48	0.43
200	0.84	1.60	0.09	0.36

Perchlorate Stability Study

The perchlorate stability study was designed to mimic conditions during a normal animal testing study. Exposure of the perchlorate stability solutions to light, temperature and humidity was carefully controlled and monitored. The temperature and relative humidity of the animal room were between 70 to 72°F and 60 to 65% relative humidity. The light/dark cycle was set at 12 hour intervals, except for day 11 of the study. On day 11 of the study, the perchlorate stability solutions were exposed to 10 hours of light and 14 hours of dark due to a power outage. The intensity of incident light on the top left, top right, center, bottom left and bottom right of the set of animal cages was measured on days 10, 27, 41, 54, and 102. The results are shown in Table 3.3. As expected, stability solutions located on the top of the animal cages, closest to the light source, were consistently exposed to more light than those on the bottom of the animal cages. For a given location on the set of animal cages, variability in light intensity with respect to time, as measured by percent coefficient of variation (% CV), was less than 6%, indicating little or no change in light intensity. Daily rotation of the animal cages ensured that throughout the entire study, stability solutions were exposed to equal amounts of light. Respectively, the daily mean light intensity for days 10, 27, 41, and 54 were 34.8, 35.6, 35.7, and 32.9. The average, standard deviation and %CV for the daily mean light intensity were 34.7 fc, 1.10 fc and 3.7% CV, respectively.

Table 3.3. Incident light intensity measurements.

Day	Top Left	Top Right	Middle	Bottom Left	Bottom Right
	(fc)	(fc)	(fc)	(fc)	(fc)
10	37.5	38.2	35.2	31.0	32.0
27	37.3	37.7	39.0	30.0	33.8
41	38.7	38.5	37.3	30.0	34.1
54	37.5	35.7	33.7	28.4	29.4
Avg.	37.8	37.5	36.3	29.9	32.3
Std. Dev.	0.5	1.0	1.8	0.8	1.7
%CV	1.6%	2.6%	4.9%	2.8%	5.4%

The stability data of 0.05 and 200 µg/mL stability solutions in clear and amber bottles are listed in Appendix A. Because the stability solutions were pooled on day 109 of the study, only

two data points are shown. Concentrations of the stability solutions were measured twice by IC as described in Section II. Each ion chromatogram was examined in detail and the formation of nitrate was not observed in any of the stability solutions.

A one way analysis of variance (ANOVA) analysis, shown on the bottom of each table, was employed to examine the differences of within group (intra-day) and between groups (inter-day) concentration variations. As shown in Appendix A, three categories of sums of squares (*SS*) are presented in the ANOVA summary report, along with the degrees of freedom (*df*) for the between and within variance. The mean square (*MS*) and the test for homogeneity of variance (*F*-ratio) were calculated from *SS* and *df* by the following equations: $MS = SS/df$ and *F*-ratio = between *MS*/ within *MS*. The *F critical* values at 0.05 rejection level (α) were obtained from Reference 13. As compared to the appropriate F-critical values, the small F-values (test of homogeneity of variance) for all four sets of data indicated that ammonium perchlorate in aqueous solution at 0.05 and 200 µg/mL is stable for 109 days. At a given level, no trend was observed in the perchlorate concentration, as some might expect an increasing trend due to evaporation. Furthermore, no significant perchlorate concentration difference was noted between the solutions stored in amber and clear water bottles at a given concentration. Since the amber bottles are less impermeable to light and UV radiation, the results indicated that average 12-hour daily exposure to light does not lead to the degradation of perchlorate in reagent water.

Concentration Verification

The results of concentration verification analysis for Argus Research Laboratories, Inc., and Springborn Laboratories, Inc., are presented in Appendices B and C, respectively. Date of formulation preparation and analysis date are clearly denoted. If possible, duplicate sample analysis was performed for every set of samples. If performed, duplicates were analyzed at least once every ten samples. As shown in Appendices B and C, the very low percent difference between the concentration of duplicate samples ensures the method is reproducible. Spike recovery analysis at either 0.05 or 200 µg/mL was performed for every set of samples. The percent spike recovery was consistently between +/-10%, indicating acceptable method accuracy. The difference between the nominal and measured concentration was within 90 to 110%. No nitrate was found in any of the formulations.

SECTION IV: DISCUSSION AND CONCLUSIONS

A sensitive IC method for the analysis of perchlorate and nitrate has been developed and optimized to support the stability study of ammonium perchlorate in water. The method detection limit for perchlorate and nitrate is 0.005 µg /mL (5 ppb). The sensitivity of the method is comparable to existing IC [14-17] and CE [18, 19] methods. This optimized IC method is far more sensitive and selective than gravimetry [20-23], UV-spectrophotometry [24-38], ion selective electrode [39-42] and flame atomic absorption spectroscopy [43] methods. Methods such as those of California Department of Health Services (CDHS) are not as robust as this IC method; the CDHS can not detect nitrate. The method is linear from MDL to 4000 x MDL and is demonstrated to have acceptable precision and accuracy.

Since perchlorate is a strong oxidizing agent, it was thought that the stability of perchlorate may be limited. [1] A stability study of ammonium perchlorate in reagent water was conducted at constant temperature and humidity. Ammonium perchlorate in reagent water was found to be stable in the presence of light at two concentrations (0.05 and 200 µg/mL). Detailed examination of the ion chromatograms did not show the formation of nitrate in the stability solution for up to 109 days of storage. Due to the very low MDL, we believe that any degradation of ammonium perchlorate would have been detected within this time period. Stability solutions for a given concentration level and bottle type were pooled and analyzed at day 145. The results of the pooled stability solutions indicated that perchlorate appears to be still stable.

Concentration verification of dosing solutions was performed for samples received from Argus Research Laboratories, Inc., and Springborn Laboratories, Inc. The concentration of the dosing solutions was determined by IC. Measured concentrations agreed well with the nominal concentrations.

IC was used to aggressively monitor the possible presence of nitrate in the dosing solution because nitrate is an interfering anion commonly found in rural ground water supply. At the method detection limit of 0.005 µg/mL, no nitrate was found in the dosing solutions.

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SECTION VI: QUALITY ASSURANCE

The study, "Stability and Concentration Verification of Ammonium Perchlorate Dosing Solutions," was conducted to be in compliance with the Environmental Protection Agency's Good Laboratory Practices Standards, 40 CFR 792.

The data, notebook, and Investigators Report for this study were inspected by the Quality Assurance Unit. Data for light intensity measurement were not included in this review. These data were collected by the auditor in his capacity as Unit Safety Representative for AFRL/HEST, Toxicology Branch. Results of the inspections were reported directly to the Investigator.

DATE OF INSPECTION	ITEM INSPECTED
March 16, 17, 1998	Data, Notebook
March 19, 1998	Data, Notebook
April 30, 1998	Report

The Quality Assurance Unit has determined through review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretations presented in this Final Report.

M G Schneider

M. G. Schneider
QA Coordinator
Toxic Hazards Research

Date April 30, 1998

APPENDIX A. STABILITY DATA

0.05 µg/mL PERCHLORATE SOLUTION IN CLEAR BOTTLE						
	Day 7	Day15	Day36	Day50	Day61	Day109
Bottle A	0.048	0.050	0.051	0.050	0.050	0.050
Bottle B	0.050	0.051	0.048	0.049	0.051	
Bottle C	0.051	0.050	0.048	0.051	0.051	0.050
Bottle D	0.052	0.049	0.050	0.050	0.049	
ANOVA: Single Factor, $\alpha = 0.05$						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Day 7	4	0.201	0.050	2.9E-06		
Day15	4	0.200	0.050	6.7E-07		
Day36	4	0.196	0.049	1.9E-06		
Day50	4	0.201	0.050	5.2E-07		
Day61	4	0.201	0.050	9.2E-07		
Day109	2	0.100	0.050	0		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.51E-06	5	9.02E-07	0.69858	0.63236	2.85241
Within Groups	2.07E-05	16	1.29E-06			
Total	2.52E-05	21				

200 µg/mL PERCHLORATE SOLUTION IN CLEAR BOTTLE						
	Day 7	Day15	Day36	Day50	Day61	Day109
Bottle A	198.44	200.00	206.66	196.67	201.39	200.67
Bottle B	199.27	199.57	200.89	200.45	202.81	
Bottle C	204.55	200.16	201.89	199.59	196.92	199.58
Bottle D	207.95	199.90	199.24	202.36	203.11	
ANOVA: Single Factor, $\alpha = 0.05$						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Day 7	4	810.2	202.553	20.2702		
Day15	4	799.6	199.909	0.0607		
Day36	4	808.7	202.169	10.1468		
Day50	4	799.1	199.768	5.5960		
Day61	4	804.2	201.057	8.1864		
Day109	2	400.2	200.123	0.5962		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	27.493	5	5.49861	0.65962	0.65905	2.85241
Within Groups	133.376	16	8.33601			
Total	160.869	21				

0.05 μg/mL PERCHLORATE SOLUTION IN AMBER BOTTLE						
	Day 7	Day15	Day36	Day50	Day61	Day109
	0.055	0.050	0.049	0.050	0.050	0.052
	0.048	0.049	0.050	0.049	0.049	0.051
	0.052	0.051	0.048	0.049	0.050	
ANOVA: Single Factor, $\alpha = 0.05$						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Day 7	3	0.155	0.052	1.2E-05		
Day15	3	0.155	0.050	1.0E-06		
Day36	3	0.147	0.049	1.1E-06		
Day50	3	0.148	0.049	7.9E-07		
Day61	3	0.149	0.050	4.4E-07		
Day109	2	0.103	0.052	5.0E-07		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.83E-05	5	3.66E-06	1.26043	0.34664	3.20388
Within Groups	3.19E-05	11	2.90E-06			
Total	5.02E-05	16				

200 µg/mL PERCHLORATE SOLUTION IN AMBER BOTTLE						
	Day 7	Day15	Day36	Day50	Day61	Day109
	202.41	200.00	207.04	202.00	199.42	200.67
	197.39	203.19	200.89	200.25	200.78	199.58
	199.10	200.31	200.41	199.50	197.66	
ANOVA: Single Factor, $\alpha = 0.05$						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Day 7	3	598.9	199.633	6.5134		
Day15	3	603.5	201.165	3.0923		
Day36	3	608.3	202.779	13.7003		
Day50	3	601.8	200.583	1.6443		
Day61	3	597.9	199.284	2.4465		
Day109	2	400.2	200.123	0.5962		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	23.651	5	4.73016	0.93937	0.49285	3.20388
Within Groups	55.389	11	5.03542			
Total	79.0404	16				

APPENDIX B. CONCENTRATION VERIFICATION ANALYSIS FOR ARGUS LABORATORY, INC.

Sponsor's Study No. 7757A210-1096-25F				
SLI Vehicle	**R.O. D.I. Water**			
Protocol No.	**1613-002**			
Prep Date	**9/22/97**			
Test Article No.	**S97.001.3455**			
Analysis Date	**9/23/97**			
		Measured		
	Nominal	Perchlorate	Perchlorate	Percent
	Concentrations	Concentrations	MDL	Difference
Identifiers	(µg/mL)	(µg /mL)	(µg /mL)	(%)
1613-002 A	0.000	0	0.005	0.00%
1613-002 B	0.800	0.778	0.005	2.75%
1613-002 C	7.600	7.504	0.005	1.26%
1613-002 D	22.800	21.752	0.005	4.60%
1613-002 E	75.800	76.864	0.005	1.40%
1613-002 F	50000.000	49996.000	0.005	0.01%
QA/QC Data				
	Nominal	Measured		
	Concentrations	Perchlorate		
	(µg /mL)	Concentrations		
		(µg /mL)		
Control #1	20	20.171		
Control #2	20	19.3		
Average	20	19.7355		
% Difference		4.32%		
% Recovery		**98.68%**		
1613-002B	0.800	0.778		
1613-002B Duplicate	0.800	0.776		
% Difference		**0.26%**		
1613-002B	0.8	0.778		
1613-002B, 20ppm Spike	20.8	20.752		
% Spike Recovery		**96.25%**		

Sponsor's Study No. 7757A210-1096-25F				
SLI Vehicle	**R.O. D.I. Water**			
Protocol No.	**1613-002**			
Prep Date	**10/6/97**			
Test Article No.	**S97.001.3455**			
Analysis Date	**10/6/97**			
		Measured		
	Nominal	Perchlorate	Perchlorate	Percent
	Concentrations	Concentrations	MDL	Difference
Identifiers	(µg /mL)	(µg /mL)	(µg /mL)	(%)
1613-002 A	0.000	0	0.005	0.00%
1613-002 B	0.800	0.812	0.005	1.50%
1613-002 C	7.600	7.920	0.005	4.21%
1613-002 D	22.800	21.547	0.005	5.50%
1613-002 E	75.800	74.463	0.005	1.76%
QA/QC Data		Measured		
	Nominal	Perchlorate		
	Concentrations	Concentrations		
	(µg /mL)	(µg /mL)		
Control #1	20	19.856		
Control #2	20	19.856		
Average	20	19.856		
% Difference		0.00%		
% Recovery		**99.28%**		
1613-002B	0.800	0.812		
1613-002B Duplicate	0.800	0.813		
% Difference		**0.12%**		
1613-002B	0.8	0.812		
1613-002B, 20ppm Spike	20.8	21.12		
% Spike Recovery		**96.16%**		

Sponsor's Study No. 7757A210-1096-25F				
SLI Vehicle	**R.O. D.I. Water**			
Protocol No.	**1613-002**			
Prep Date	**10/27/97**			
Test Article No.	**S97.001.3455**			
Analysis Date	**10/28/97**			
		Measured		
	Nominal	Perchlorate	Perchlorate	Percent
	Concentrations	Concentrations	MDL	Difference
Identifiers	(µg /mL)	(µg /mL)	(µg /mL)	(%)
1613-002 A	0.000	0	0.005	0.00%
1613-002 J	0.580	0.575	0.005	0.86%
1613-002 K	5.800	5.758	0.005	0.72%
1613-002 L	17.400	17.394	0.005	0.03%
1613-002 M	58.000	59.026	0.005	1.77%
1613-002 N	50000.000	50395.000	0.005	0.79%
QA/QC Data				
	Nominal	Measured		
	Concentrations	Perchlorate		
	(µg /mL)	Concentrations		
		(µg /mL)		
Control #1	20	20.047		
Control #2	20	20.047		
Average	20	20.047		
% Difference		0.00%		
% Recovery		**100.24%**		

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APPENDIX C. CONCENTRATION VERIFICATION ANALYSIS FOR SPRINGBORN LABORATORIES, INC.

SLI Study No. 3455.1				
SLI Vehicle	**R.O. D.I. Water**			
Prep Date	**9/2/97**			
Test Article No.	**S97.001.3455**			
Analysis Date	**9/2/97**			
		Measured		
	Nominal	Perchlorate	Perchlorate	Percent
	Concentrations	Concentrations	MDL	Difference
Identifiers	(µg /mL)	(µg /mL)	(µg /mL)	(%)
Control A	0.000	ND	0.005	0.00%
Gr. 2 M A	0.083	0.081	0.005	2.41%
Gr. 2 F A	0.074	0.072	0.005	2.70%
Gr. 3 M A	0.470	0.043	0.005	2.38%
Gr. 3 F A	0.037	0.038	0.005	2.70%
Gr. 4 M A	1.670	1.689	0.005	1.14%
Gr. 4 F A	1.470	1.534	0.005	4.35%
Gr. 5 M A	8.330	8.043	0.005	3.45%
Gr. 5 F A	7.370	7.138	0.005	3.15%
Gr. 6 M A	83.300	82.477	0.005	0.99%
Gr. 6 F A	73.700	72.627	0.005	1.46%
50 mg/mL	50000.000	50230	1.250	0.46%
QA/QC Data				
	Nominal	Measured		
	Concentrations	Perchlorate		
	(µg /mL)	Concentrations		
		(µg /mL)		
Control #1	20	19.3		
Control #2	20	19.3		
Average	20	19.3		
% Difference		0.00%		
% Recovery		**96.50%**		
Gr. 4M A	1.667	1.689		
Gr. 4M B Duplicate	1.667	1.684		
% Difference		**0.30%**		
Gr. 2F A	0.074	0.072		
Gr. 2F B+20ppm spike	20.074	19.375		
% Spike Recovery		**99.63%**		

SLI Study No. 3455.1				
SLI Vehicle	**R.O. D.I. Water**			
Prep Date	**9/22/97**			
Test Article No.	**S97.001.3455**			
Analysis Date	**9/22/97**			
		Measured		
	Nominal	Perchlorate	Perchlorate	Percent
	Concentrations	Concentrations	MDL	Difference
Identifiers	(µg /mL)	(µg /mL)	(µg /mL)	(%)
Gr. 1 M/F	0.000	0.000	0.005	0.00%
Gr. 2 M A	0.093	0.091	0.005	2.15%
Gr. 2 F A	0.081	0.079	0.005	2.47%
Gr. 3 M A	0.477	0.454	0.005	4.82%
Gr. 3 F A	0.390	0.350	0.005	9.54%
Gr. 4 M A	1.813	1.715	0.005	5.41%
Gr. 4 F A	1.647	1.621	0.005	1.58%
Gr. 5 M A	9.100	9.115	0.005	0.16%
Gr. 5 F A	7.800	7.575	0.005	2.88%
Gr. 6 M A	92.000	89.313	0.005	2.92%
Gr. 6 F A	68.333	68.078	0.005	0.37%
50 mg/mL, 1:250 Dil	50000	49200	1.250	1.60%
QA/QC Data				
	Nominal	Measured		
	Concentrations	Perchlorate		
	(µg /mL)	Concentrations		
		(µg /mL)		
Control #1	20	20.171		
Control #2	20	20.275		
Average	20	20.223		
% Difference		0.52%		
% Recovery		**101.12%**		
Gr. 2M A	0.093	0.091		
Gr. 2M B Duplicate	0.093	0.091		
% Difference		**0.00%**		
Gr. 2F A	0.081	0.079		
Gr. 2F B+20ppm spike	20.081	20.187		
% Spike Recovery		**99.61%**		

SLI Study No. 3455.1				
SLI Vehicle	**R.O. D.I. Water**			
Prep Date	**10/6/97**			
Test Article No.	**S97.001.3455**			
Analysis Date	**10/7/97**			
		Measured		
	Nominal	Perchlorate	Perchlorate	Percent
	Concentrations	Concentrations	MDL	Difference
Identifiers	(µg /mL)	(µg /mL)	(µg /mL)	(%)
Gr. 1 M/F	0.000	0.000	0.005	0.00%
Gr. 2 M A	0.105	0.106	0.005	0.95%
Gr. 2 F A	0.083	0.084	0.005	1.20%
Gr. 3 M A	0.552	0.572	0.005	3.62%
Gr. 3 F A	0.419	0.407	0.005	2.86%
Gr. 4 M A	2.142	2.254	0.005	5.23%
Gr. 4 F A	1.533	1.560	0.005	1.74%
Gr. 5 M A	10.292	10.278	0.005	0.14%
Gr. 5 F A	8.500	8.437	0.005	0.74%
Gr. 6 M A	109.167	103.055	0.005	5.60%
Gr. 6 F A	80.000	81.016	0.005	0.85%
50 mg/mL, 1:250 Dil	50000	50900.000	1.250	1.80%
QA/QC Data				
	Nominal	Measured		
	Concentrations	Perchlorate		
	(µg /mL)	Concentrations		
		(µg /mL)		
Control #1	20	19.856		
Control #2	20	19.856		
Average		19.856		
% Difference		0.00%		
% Recovery		**99.28%**		
Gr. 2F A	0.083	0.084		
Gr. 2F B Duplicate	0.083	0.084		
% Difference		**0.00%**		
Gr. 2M A	0.105	0.106		
Gr. 2M B+20ppm spike	20.105	20.187		
% Spike Recovery		**99.47%**		

SLI Study No.	**3455.1**			
SLI Vehicle	**R.O. D.I. Water**			
Prep Date	**10/28/97**			
Test Article No.	**S97.001.3455**			
Analysis Date	**10/28/97**			
		Measured		
	Nominal	Perchlorate	Perchlorate	Percent
	Concentrations	Concentrations	MDL	Difference
Identifiers	(µg /mL)	(µg /mL)	(µg /mL)	(%)
Gr. 1 M/F	0.000	0.000	0.005	0.00%
Gr. 2 M A	0.114	0.114	0.005	0.00%
Gr. 2 F A	0.078	0.079	0.005	1.28%
Gr. 3 M A	0.598	0.581	0.005	2.84%
Gr. 3 F A	0.398	0.398	0.005	0.00%
Gr. 4 M A	2.322	2.406	0.005	3.62%
Gr. 4 F A	1.589	1.604	0.005	0.94%
Gr. 5 M A	11.667	11.630	0.005	0.32%
Gr. 5 F A	8.375	8.487	0.005	1.34%
Gr. 6 M A	114.583	115.253	0.005	0.58%
Gr. 6 F A	81.667	82.516	0.005	1.04%
50 mg/mL,1:250 Dil	50000	50200.000	1.250	0.40%
QA/QC Data				
	Nominal	Measured		
	Concentrations	Perchlorate		
	(µg /mL)	Concentrations		
		(µg /mL)		
Control #1	20	20.047		
Control #2	20	20.013		
Average		20.03		
% Difference		0.17%		
% Recovery		**100.15%**		
Gr. 2F A	0.078	0.079		
Gr. 2F B Duplicate	0.083	0.079		
% Difference		**0.00%**		
Gr. 2M A	0.114	0.114		
Gr. 2M B+20ppm spike	20.114	20.134		
% Spike Recovery		**99.43%**		

SLI Study No.	**3455.1**			
SLI Vehicle	**R.O. D.I. Water**			
Prep Date	**11/24/97**			
Test Article No.	**S97.001.3455**			
Analysis Date	**11/24/97**			
		Measured		
	Nominal	Perchlorate	Perchlorate	Percent
	Concentrations	Concentrations	MDL	Difference
Identifiers	(µg /mL)	(µg /mL)	(µg /mL)	(%)
Gr. 1 M/F	0.000	0.000	0.005	0.00%
Gr. 2 M A	0.126	0.126	0.005	0.00%
Gr. 2 F A	0.093	0.092	0.005	1.08%
Gr. 3 M A	0.694	0.686	0.005	1.15%
Gr. 3 F A	0.438	0.436	0.005	0.46%
Gr. 4 M A	2.775	2.704	0.005	2.56%
Gr. 4 F A	2.000	1.969	0.005	1.56%
Gr. 5 M A	13.458	13.258	0.005	1.49%
Gr. 5 F A	9.583	9.493	0.005	0.94%
Gr. 6 M A	129.167	130.086	0.005	0.71%
Gr. 6 F A	90.417	91.477	0.005	1.17%
50 mg/mL,1:250 Dil	50000	50700.000	1.250	1.40%
QA/QC Data				
	Nominal	Measured		
	Concentrations	Perchlorate		
	(µg /mL)	Concentrations		
		(µg /mL)		
Control #1	20	20.094		
Control #2	20	20.013		
Average		20.0535		
% Difference		0.40%		
% Recovery		**100.27%**		
Gr. 2F A	0.093	0.092		
Gr. 2F B Duplicate	0.093	0.092		
% Difference		**0.00%**		
Gr. 2M A	0.126	0.126		
Gr. 2M B+20ppm spike	20.126	20.177		
% Spike Recovery		**99.38%**		